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ScienceDirect
Veterinary Microbiology 132 (2008) 65–73
veterinary microbiology
www.elsevier.com/locate/vetmic

Experimental infection of rabbits with ovine herpesvirus 2 from sheep nasal secretions

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Received 18 January 2008; received in revised form 21 April 2008; accepted 25 April 2008

Abstract

Malignant catarrhal fever (MCF) is a generally fatal disease that primarily occurs in ruminants and is caused by a group of gammaherpesviruses. Outside of Africa MCF is mainly caused by ovine herpesvirus 2 (OvHV-2) which is carried subclinically by sheep. Cell-free virus is present in nasal secretions of shedding sheep and aerosol is the primary mode of transmission. Although OvHV-2 has never been propagated in vitro, experimental infection involving intranasal nebulization with nasal secretions from shedding sheep has been used to induce MCF in cattle and bison. This method of inoculation has never been tested in rabbits, which are the primary small animal model. The objectives of this study were to determine whether rabbits become infected with OvHV-2 after intranasal nebulization with cell-free virus from sheep nasal secretions and whether they develop MCF with consistent gross and histologic lesions. Five of eight rabbits became infected, showed clinical signs and developed histologic lesions typical of MCF including multisystemic vasculitis and perivascular lymphoid accumulation. These lesions are similar to those reported in rabbits infected by intravenous injection with tissues from clinically affected animals containing cell-associated virus. Viral DNA and mRNA transcripts of a structural viral protein were present in tissues from affected rabbits suggesting that viral replication occurred, although the significance in terms of pathogenesis is unknown. This work demonstrates that OvHV-2 infection of rabbits by intranasal nebulization is a potentially useful model that mimics the natural route of infection and may be used to study viral replication and pathogenesis.
Published by Elsevier B.V.

Keywords: Ovine herpesvirus 2; Malignant catarrhal fever; Rabbits; Experimental infection

1. Introduction

Malignant catarrhal fever (MCF) is a gammaherpesviral disease of ruminants that involves multisystemic lymphoproliferation, vasculitis and epithelial

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0378-1135/$ – see front matter. Published by Elsevier B.V.
doi:10.1016/j.vetmic.2008.04.035

necrosis and is generally fatal in clinically susceptible hosts. The disease is caused primarily by ovine herpesvirus 2 (OvHV-2) and alcelaphine herpesvirus 1 (AlHV-1), which are carried subclinically by sheep and wildebeest, respectively. Sheep-associated MCF (SA-MCF) occurs worldwide while the wildebeest-associated form (WA-MCF) is restricted to Africa and zoological parks housing wildebeest. The disease is economically important to various livestock industries including bison ranching in North America (Schultheiss et al., 2000; Berezowski et al., 2005; Li et al., 2006), cattle ranching in Africa (Plowright, 1990) and deer ranching in New Zealand (Audige et al., 2001) and has caused mortality in numerous zoos and wild animal parks around the world (Hatkin, 1980; Meteyer et al., 1989; Li et al., 1999).

Ovine herpesvirus 2 has eluded numerous attempts at in vitro propagation which has hampered research advances due to the lack of a system for producing consistent, large quantities of virus for experimental infection. However, a variety of research techniques are now available, including serology, OvHV-2 specific PCR and real-time PCR, that have allowed for the elucidation of the unique OvHV-2 shedding pattern in sheep and subsequent collection of infectious OvHV-2 in sheep nasal secretions (Li et al., 2001a, 2004). Intranasal nebulization with a standardized pool of nasal secretions from shedding sheep mimics the likely natural route of infection via the respiratory tract and has been used to successfully infect and induce disease in sheep, cattle and bison (Taus et al., 2005, 2006; Li et al., 2005; O'Toole et al., 2007). Dose response analysis from these studies indicates that cattle are relatively resistant to SA-MCF and require prohibitively large quantities of virus to induce clinical disease. While bison are highly susceptible to experimentally induced SA-MCF, they are expensive to purchase and maintain and require special handling facilities. For these reasons, a small animal model involving a natural route of infection is desirable.

Wildebeest-associated MCF has been experimentally induced in rabbits, hamsters, guinea-pigs and rats by intracranial (IC), intraperitoneal (IP), intravenous (IV) and intracardiac injection of cultured cells (Kalunda et al., 1981; Jacoby et al., 1988). However, SA-MCF has only been successfully induced in rabbits and hamsters by injection (IP, IV or IC) of presumably cell-associated virus in non-cultured tissue suspension from clinically affected animals or non-productively infected cell cultures (Buxton and Reid, 1980; Reid et al., 1986; Jacoby et al., 1988; Anderson et al., 2007). Both rabbits and hamsters develop gross and histologic lesions consistent with MCF including multisystemic interstitial and perivascular lymphoproliferation and to a lesser degree vasculitis (Buxton et al., 1984, 1988). However, since the late 1980s, injection of rabbits (rather than hamsters) with infected tissue suspension or non-productively infected lymphocyte cell lines (Anderson et al., 2007) has been used exclusively as the small animal model for study of SA-MCF. In order to study the true dynamics of viral infection, replication, dissemination and pathogenesis a model that involves a natural route of transmission is ideal. For this study we sought to determine if rabbits become infected with OvHV-2, show clinical signs and develop lesions consistent with MCF when inoculated by intranasal nebulization with sheep nasal secretions containing cell-free OvHV-2 and to compare the findings with experiments involving intraperitoneal injection with similar inoculum.

2. Materials and methods

2.1. Animals

Sixteen 4-month-old male, New Zealand white rabbits were obtained from Myrtle's rabbitry (Thompson Station, TN 37179) and were maintained at Washington State University, Pullman, WA in accordance with an approved animal care and use protocol. Blood was drawn from an aural artery of each rabbit three times at 1–2-week intervals prior to inoculation and all rabbits were seronegative for antibody against MCF-group viruses by competitive inhibition enzyme-linked immunosorbent assay (cELISA) and no OvHV-2 DNA was detected by PCR (see Section 2.4). After an acclimation period of 1 month, each rabbit was given three consecutive daily doses of 5 mg/kg enrofloxacin (Baytril) by intramuscular injection into the caudal thigh muscles to prevent infection by potential bacterial pathogens present in the sheep nasal secretions.

2.2. Nasal secretion inoculum

Ovine nasal secretions were collected from a group of 6–9-month old lambs at times of peak shedding as described previously (Li et al., 2001a, 2004; Kim et al., 2003). Briefly, cotton tipped swabs were inserted into the nasal cavity and removed after 2 min and placed in cold phosphate buffered saline. The solution was clarified by low speed centrifugation and secretions were pooled and stored in liquid nitrogen until use. The infectivity of OvHV-2 in the pooled secretions was characterized by nebulization of sheep and between 10^2 and 10^3 DNA copies was determined to be the minimum sheep infectious dose.

2.3. Inoculation of rabbits

A total of 16 rabbits were inoculated with 2 ml of sheep nasal secretions from the same pooled batch that either contained 10^7 OvHV-2 DNA copies (test animals) or were collected from OvHV-2 negative sheep (negative controls). Group A included eight test animals and two control animals that were inoculated by intranasal nebulization using a cone that covered the nose and mouth as previously described for sheep (Li et al., 2004). Group B included four test animals and two negative control rabbits that were inoculated by intraperitoneal injection.

2.4. Antemortem sample collection and assays

Two to three milliliters of EDTA anticoagulated blood were collected from an aural artery of each rabbit twice weekly for 4 weeks, once a week for an additional 8 weeks and then monthly until termination of the experiment at 18 weeks (125 days) post-inoculation (PI). Plasma was separated and tested for antibody against MCF group viruses using the cELISA (Li et al., 2001b). Semi-nested PCR specific for OvHV-2 was used to detect OvHV-2 DNA in peripheral blood leukocytes (PBL) (Baxter et al., 1993; Li et al., 2004). Body temperature was taken every 2–3 days for 8 weeks and weekly thereafter.

2.5. Postmortem sample collection and analysis

Rabbits were euthanized within 48 h of the onset of pyrexia (>104 °F) or clinical signs. All remaining animals were euthanized at the termination of the experiment. Complete necropsies were performed on all group A test rabbits, one test rabbit from group B (#13) and one negative control rabbit from each group (#1 and #11). All necropsies were performed within 1 h of euthanasia and included collection of a full set of tissues in 10% neutral buffered formalin for histopathology and fresh samples of mesenteric lymph node, spleen, lung, liver and urinary bladder that were snap frozen in liquid nitrogen for DNA and RNA analysis. Real-time PCR was performed as described by Hussy et al. (2001) with a few modifications (Li et al., 2004) and was performed using 50 ng total DNA from PBL and tissues. The real-time PCR assay has been validated for use in diagnosis of MCF in clinically affected animals with a minimum detection limit of 50 DNA copies (Traul et al., 2007).

Reverse transcriptase PCR (RT-PCR) was performed as previously described (Cunha et al., 2007) on total RNA extracted from approximately 100 mg homogenized tissue. The primers used amplify a 465 base pair region of OvHV-2 ORF 25, an OvHV-2 gene transcribed late during the viral replication cycle.

3. Results

3.1. OvHV-2 infection and clinical signs

Ovine herpesvirus 2 DNA was detected in PBL of five of the eight test animals (62.5%) in group A between 18 and 38 days PI (Table 1). All five of these rabbits also became seropositive for antibody against the MCF group viruses between 25 and 34 days PI, confirming infection. All five seropositive and PCR positive rabbits in group A developed pyrexia 25–38 days PI. These five rabbits also developed clinical signs varying from mild depression with retained appetite to severe depression with complete anorexia. No other clinical signs were noted. One of the test animals in group A (#9) became severely depressed and anorexic on day 114 PI and was euthanized although no virus specific antibody could be detected in plasma and OvHV-2 DNA could not be identified in PBL or tissues by PCR (Table 2). There were no other indications of disease in this rabbit (see Section 3.3) and the cause of the clinical signs is unknown. The remaining two test animals in group A, the four test animals in group B and

Table 1
Clinical and laboratory findings in rabbits inoculated with OvHV-2 from sheep nasal secretions

Animal ID	Group[a]	Clinical outcome	Onset pyrexia[b]	Euthanasia[b]	Antibody[b]	DNA[b]	Real-time PCR (PBL)[c]
1	A (NC)	Healthy	None	End	–	–	na
2	A (NC)	Healthy	None	End	–	–	na
3	A	MCF[d]	25	27	25	25	44,400
4	A	Healthy	None	End	–	–	na
5	A	MCF	38	38	34	25	35,700
6	A	MCF	27	28	25	18	5,390
7	A	Healthy	None	End	–	–	na
8	A	MCF	33	34	34	38	185
9	A	Unknown	None	112	–	–	na
10	A	MCF	27	28	28	18	4,990
11	B (NC)	Healthy	None	End	–	–	na
12	B (NC)	Healthy	None	End	–	–	na
13	B	Healthy	None	End	–	–	na
14	B	Healthy	None	End	–	–	na
15	B	Healthy	None	End	–	–	na
16	B	Healthy	None	End	–	–	na

[a] Group A inoculated with OvHV-2 by intranasal nebulization and group B by intraperitoneal injection. NC = negative control.
[b] Results of experimental infection reported as days post-inoculation (PI) of first detection (pyrexia, antibody, DNA) or euthanasia. Euthanasia was performed within 48 h of onset of pyrexia (>104 °F), except where ''end' indicates the animal was healthy until euthanasia at the end of the experiment (125 days PI). MCF virus group antibody detected in plasma by cELISA and OvHV-2 DNA detected by nested PCR on buffy coat cells. (–) = not detected.
[c] Real-time PCR results from peripheral blood leukocytes (PBL) collected on the day of euthanasia and reported as OvHV-2 genome copies/50 ng total DNA. na = not tested.
[d] ''MCF'' is defined as rabbit with one or more clinical signs (pyrexia, anorexia, lethargy) and consistent histologic lesions.

all control animals remained healthy and tested negative for virus specific antibody and OvHV-2 DNA in PBL samples until the end of the experiment.

3.2. OvHV-2 genome copy number and transcripts

All PBL samples in which OvHV-2 DNA was detected by nested PCR were also tested for OvHV-2 genome copy number by real-time PCR. Between 185 and 44,400 OvHV-2 DNA copies per 50 ng total DNA were detected in the PBL of the five MCF affected rabbits in group A on the day of euthanasia (Table 1). Tissues including mesenteric lymph node, lung, spleen and urinary bladder from the five affected rabbits were also tested by real-time PCR and all but one tissue sample (urinary bladder from rabbit 8) contained between 373 and 111,000 OvHV-2 DNA copies per 50 ng DNA (Table 2). We recently reported

Table 2
OvHV-2 DNA and ORF25 transcripts in tissues of rabbits with experimental MCF

Animal ID	Lymph node			Lung			Spleen			Urinary bladder		
	DNA[a]	RNA[b]	Lesions[c]	DNA	RNA	Lesions	DNA	RNA	Lesions	DNA	RNA	Lesions
3	111,000	+	L	66,900	+	V	39,100	+	L	373	+	None
5	56,000	+	L	20,100	+	V	61,200	+	L	1660	+	L
6	8,580	+	L	28,300	+	V, N	3,800	+	L	529	+	L
8	17,500	+	L	705	+	V	4,720	+	L	<50	+	None
10	972	+	L	8,320	+	V	7,610	+	L	1370	+	Cystitis

[a] Reported as number of OvHV-2 genome copies/50 ng total DNA as determined by real-time PCR.
[b] Reverse-transcriptase PCR (RT-PCR) for ORF25 transcripts performed using total RNA from 100 mg homogenized tissue. (+) = transcript detected.
[c] Lesions in selected tissues. L = lymphoid hyperplasia, V = lymphoid accumulation with vasculitis, N = necrosis.

the presence of ORF25 transcripts in cells from nasal secretions and respiratory tract tissues (mainly turbinate) from shedding sheep and the absence of these transcripts in similar samples from infected but non-shedding sheep indicating an association between this transcript and viral replication and shedding (Cunha et al., 2007). Therefore, rabbit tissue samples were tested for the presence of ORF 25 transcripts as an indicator of viral replication. Transcripts were detected in all four tissues tested from the five MCF affected group A rabbits (Table 2) and not in one unaffected group A rabbit that was tested (#4) or the group A negative control animal (#1) (data not shown).

3.3. Gross and histologic lesions

Two of the test rabbits in group A (#5 and #6) had multifocal, pinpoint, tan foci scattered randomly throughout the liver. One rabbit (#5) had a focal, 1 cm diameter mucosal ulcer in the proximal cecum that was covered by a layer of fibrin and was surrounded by a rim of hyperemia. No gross lesions were detected in any of the other test rabbits (including #9), or the negative control rabbits.

All five test rabbits in group A had multisystemic lesions including interstitial inflammation and perivascular accumulation of large lymphocytes, vasculitis and mild lymph node and splenic hyperplasia (Table 2). Inflammatory and vascular lesions were most prominent in the lung and liver (Fig. 1). In the lung, multifocal veins and a few small to medium sized arterioles were surrounded by moderate numbers of large lymphocytes mixed with a few macrophages and rare plasma cells. Lymphocytes infiltrated and disrupted the walls of scattered veins and rare arterioles in affected areas (vasculitis). Rare vessels were completely obliterated and replaced by a mixture of lymphocytes, heterophils, fibrin and cellular debris. Portal hepatitis consisted of diffuse infiltrates of moderate numbers of large lymphocytes and fewer macrophages, plasma cells and heterophils that occasionally bridged adjacent portal areas. Portal arterioles and venules were occasionally disrupted or obliterated by inflammatory cells and cellular debris. Mild to moderate biliary hyperplasia was present and rare bile ducts contained cellular debris. A few periportal and midzonal areas of acute hepatocyte necrosis consisted of a few heterophils, small clumps of fibrin and cellular debris.

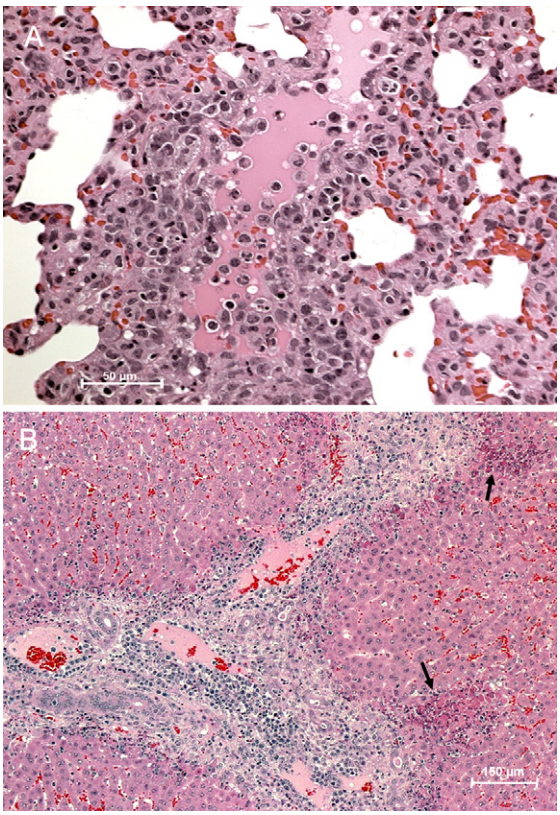

Fig. 1. Histologic lesions in a rabbit (#5) with experimentally induced MCF after intranasal nebulization with OvHV-2. (A) Lung with lymphoid accumulation and vasculitis in a medium sized vein and (B) liver with periportal vasculitis, biliary hyperplasia and multifocal hepatocyte necrosis (arrows).

Perivascular and occasional interstitial lymphocyte accumulations were also noted in the myocardium, kidney, urinary bladder, adrenal glands and thyroid gland of at least three of the five affected rabbits and in the pancreas, tongue, trachea and sclera of individual rabbits. Two rabbits had focal vasculitis in the kidney and one rabbit had a focal area of vascular necrosis in the choroid.

Lymphoid hyperplasia in lymph nodes involved mild expansion of the paracortex, and to a lesser extent the cortex, by uniform populations of large lymphocytes. Similarly, lymphoid hyperplasia in the spleen involved mildly increased numbers of large lymphocytes within the periarteriolar lymphoid sheaths, especially the mantle zone. The cecal ulcer in rabbit

#5 consisted of infiltrates of degenerate neutrophils and macrophages mixed with fibrin and cellular debris that replaced the entire tunica mucosa and submucosa and infiltrated into the underlying tunica muscularis.

4. Discussion

In the past, experimental SA-MCF has been induced in rabbits by intravenous, intraperitoneal, or intracranial injection of presumably cell-associated virus in tissue suspension from affected animals or non-productively infected cell cultures (Buxton and Reid, 1980; Reid et al., 1986; Jacoby et al., 1988; Anderson et al., 2007). However, the mode of transmission from sheep to clinically susceptible hosts under natural conditions most likely involves shedding of cell-free virus in nasal secretions of sheep and infection of susceptible hosts via the respiratory tract. This has been demonstrated by intranasal nebulization of cattle and bison with nasal secretions collected from shedding sheep (Li et al., 2004; Taus et al., 2005, 2006; O'Toole et al., 2007). To date, similar experimental inoculation of rabbits with cell-free virus by the respiratory route has not been reported. A major goal of our study was to determine if SA-MCF can be efficiently induced in rabbits by intranasal nebulization or intraperitoneal injection with nasal secretions collected from infected sheep and whether clinical signs and lesions are similar to those reported in previous rabbit studies and in other clinically susceptible hosts. None of the four rabbits that received virus by intraperitoneal injection of nasal secretions showed any evidence of infection. While the specific reason for the failure of intraperitoneal injection to induce disease is unknown, it is possible that a cell type only present in the respiratory tract is required for initial infection with cell-free virus while cell-associated virus is able to infect a circulating cell type or has a broader range of infectivity. In contrast, five of eight rabbits that were inoculated by intranasal nebulization did seroconvert, developed signs and lesions similar to previous reports of MCF in rabbits, had OvHV-2 DNA in the peripheral blood and various tissues, and had detectable OvHV-2 ORF25 transcripts in some tissues. These results clearly indicate that infection and disease can be induced in rabbits after intranasal nebulization. In addition, detection of transcripts of a structural viral protein is suggestive of viral replication. The significance of this finding in terms of disease pathogenesis is unknown and will require further detailed studies before any conclusions can be drawn.

Another goal of this study was to describe the clinical signs and lesions that occur in rabbits after intranasal nebulization with cell-free virus and to compare them with the signs and pathology reported after injection with cell-associated virus. The clinical signs including pyrexia, anorexia and lethargy observed after intranasal nebulization have also been consistently described after intravenous, intraperitoneal and intracranial inoculation (Buxton and Reid, 1980; Buxton et al., 1984) and are also similar to, but somewhat less severe than those in rabbits with experimental AlHV-1 infection and WA-MCF (Piercy, 1955; Kalunda et al., 1981). Additional clinical signs including ocular and nasal discharge and diarrhea were reported in three of fifteen rabbits that developed SA-MCF after intraperitoneal and intracranial inoculation with cell-associated OvHV-2 and in various studies involving WA-MCF in rabbits (Buxton and Reid, 1980). The reason for the lack of ocular, nasal and gastrointestinal signs in this study is unknown but very few rabbits developed these signs in the previous OvHV-2 study and the possibility of secondary infection was not addressed. The development of ocular and nasal discharge and diarrhea in WA-MCF affected rabbits occurs between 2 and 5 days after the onset of pyrexia and the absence of these signs in rabbits with SA-MCF in this and previous studies may be related to rapid clinical progression or subtle differences in pathogenesis.

The incubation period of 25–38 days (mean 29.2 ± 4.02 days) between inoculation and the onset of clinical signs (pyrexia) reported here falls within the range observed in previous studies of OvHV-2 in rabbits (3–34 days) but is on average slightly longer than the extrapolated means of 17.5 ± 6.58 days ($n = 17$) (Buxton et al., 1984) and 16.3 ± 7.20 ($n = 15$) (Buxton and Reid, 1980) from two previous studies. The slightly longer incubation after intranasal nebulization is interesting and a possible explanation is that the dynamics of the initial replication cycle in the respiratory tract is different than that which occurs in systemic circulation and may involve different host cell populations (e.g. epithelial cells vs. lymphoid

cells) or may induce a different local immune response.

Gross and histologic lesions reported in rabbits with SA-MCF after intravenous, intraperitoneal and intracranial inoculation with tissue homogenate are indistinguishable from those reported in rabbits with WA-MCF (Buxton and Reid, 1980; Buxton et al., 1984; Schock and Reid, 1996; Anderson et al., 2007). The lesions primarily involved lymphoid hyperplasia and necrosis in mesenteric lymph nodes and cecum, splenic lymphoid hyperplasia without necrosis, periportal lymphoid infiltration in the liver and scattered interstitial lymphoid infiltrates with rare, mild arteritis in the lung and kidneys. We found similar lesions in rabbits inoculated by intranasal nebulization with a few exceptions. Lymphoid hyperplasia in intranasally inoculated rabbits was not detected in the cecum, was less severe in the lymph nodes and spleen and did not consistently involve necrosis. In addition, lymphoid infiltration and vascular necrosis in the lung was more prominent and widespread in the nebulized rabbits. The reason for the lesion differences is unknown and may be related to route of infection, form of inoculum (cell-associated vs. cell-free) or differences in dose between the different studies. It is quite possible that the differences are multifactorial and that all of these factors are involved. Regardless, intranasal nebulization with cell-free virus may be the preferred method of infection because it most closely mimics what we know about the natural mode of transmission and may also more closely mimic the natural dynamics of infection and pathogenesis.

The availability of a laboratory animal for study of any infectious disease is desirable due to the ease of manipulation, more available reagents and lower cost and this is especially true for the study of SA-MCF because bison, the most susceptible known natural host, are difficult and expensive to keep and manipulate in captivity. Thus, the intranasal nebulization rabbit model may provide a useful tool for more efficiently studying certain aspects of SA-MCF infection and pathogenesis that can then be extrapolated too or tested in large animal hosts such as bison. Overall, the presence of OvHV-2 DNA and ORF25 transcripts in tissues of SA-MCF affected rabbits is consistent with the findings in similar tissues in nebulized cattle and bison (Taus et al., 2006; O'Toole et al., 2007; Cunha et al., 2007), suggesting that a similar pattern of dissemination and replication may occur in all three species after experimental infection by nebulization. However, it is important to note that there are some limitations to using the rabbit model (regardless of route of infection) in that the gross and histologic lesions in rabbits differ in important ways from those in naturally susceptible hosts. Lesions in rabbits are similar to those reported in cattle and bison experimentally infected by intranasal nebulization (Taus et al., 2006; O'Toole et al., 2007) in that interstitial lymphoid infiltration/proliferation in the liver and lung along with vasculitis are common features. However, in cattle and bison vasculitis is much more prominent and widespread and is frequently associated with marked epithelial necrosis, primarily in the gastrointestinal tract and urinary bladder. Epithelial necrosis is rare in experimentally infected rabbits and has been restricted to a few rabbits with nasal, ocular and glossal erosions, and the one rabbit in this study with a cecal ulcer. In addition, hyperplasia in lymphoid tissue such as lymph nodes, spleen and Peyer's patches occurs in rabbits, most prominently in those inoculated by injection, but is not characteristic in cattle and bison. While these differences make the use of rabbits as a model for evaluation of some aspects of MCF pathogenesis impossible, the cost benefits and convenience of using rabbits means that they may be an appropriate system for studying certain features of infection and disease that are likely common between rabbits and natural, clinically susceptible hosts.

It has been clearly demonstrated that dose plays an important role in susceptibility to infection and disease in cattle and bison and may also influence incubation period (Taus et al., 2005, 2006; O'Toole et al., 2007). To date, there is no published evidence that dose significantly influences lesion distribution or character if the animal develops SA-MCF. Thus, we think that dose could also influence the incubation period in the experimentally nebulized rabbits but it is less likely that it influences lesion development, especially when considered in light of the fact that rabbits with WA-MCF and SA-MCF induced by any method have relatively similar lesions. Previous studies in rabbits used volumes of tissue suspension, number of cells in tissue suspension, or undefined criteria to determine dose and therefore the influence of dose on the differences in lesions between our study

and the other rabbit studies is impossible to evaluate. What we can confidently evaluate is the relative susceptibility of rabbits as compared to other hosts that have been experimentally infected by intranasal nebulization. In this study, a dose of 10^7 OvHV-2 DNA copies induced disease in 5 of 8 (62.5%) of the rabbits. Thus, rabbits appear to be less resistant to SA-MCF after intranasal nebulization than cattle which require at least an order of magnitude higher dose to induce disease (one of two steers developed MCF after nebulization with 10^8 DNA copies) (Taus et al., 2006). In contrast, rabbits are much more resistant than bison which develop disease after being nebulized with a dose three orders of magnitude lower (10^4 DNA copies) (O'Toole et al., 2007). While there may be some variability in dose received by each animal due to the nebulization procedure, we consider it unlikely that this variability would consistently span orders of magnitude. At the present time, collection of infectious material from sheep is a labor intensive procedure and therefore the dose required to consistently induce disease in rabbits may somewhat limit their usefulness, especially compared to the dose required in bison which are one of the naturally affected host species of economic importance. However, in the absence of other better laboratory animal models, rabbits may provide the best alternative for carefully designed studies that would be difficult or impossible to perform in bison. This will be especially true if a system for collection of high quantities of cell-free virus ($>10^7$ DNA copies) becomes available such that disease can be reliably produced in 100% of inoculated rabbits.

Acknowledgements

This work was supported by USDA-ARS CWU 5348-32000-018-00D. We thank Jan Keller, Shirley Elias, Lori Fuller, and Emma Karel for excellent technical assistance and animal care and Lindsay Oaks and Gary Haldorson for useful technical advice.

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